

Title

Polyketide Synthase Gene from *Sorangium cellulosum*

Cross-Reference to Related Applications

The present application is a continuation-in-part of allowed U.S. patent application Serial No. 09/144,085, filed 31 Aug. 1998, which is a continuation-in-part of U.S. Patent No. 6,090,601, each of which is incorporated herein by reference.

Field of the Invention

The present invention relates to the fields of molecular biology, chemistry, and medicine.

Background of the Invention

Sorangium species produce a variety of useful polyketides, including epothilone, myxothiazole, and soraphen. U.S. Patent Nos. 5,962,290; 6,066,721; and PCT patent publication Nos. 98/49315; 99/0398600/24907; 00/31247; and 00/44717 describe methods for making novel polyketides by combining portions of two or more polyketide synthase (PKS) genes to create novel genes that encode a hybrid PKS and by providing synthetic biosynthesis intermediates to modified PKS enzymes. There remains a need for new polyketide synthase genes for use in the preparation of hybrid PKS enzymes and the polyketides produced by such hybrid enzymes. The present invention meets that need by providing recombinant DNA compounds that comprise all or a portion of a PKS gene from *Sorangium cellulosum*.

Summary of the Invention

The present invention provides recombinant DNA vectors and host cells that comprise the *tmbA* genes of *Sorangium cellulosum* or fragments of those genes.

These and other aspects of the invention are described in more detail in the following description and claims set forth below.

Brief Description of the Figures

Figure 1 provides a physical map of the *tmbA* gene cluster and an alignment of the cosmids of the invention (34-7, 28-26, and 14H12) that comprise the *tmbA* gene cluster genes and gene fragments. The PKS genes are designated *tmbA*, *tmbB*, and *tmbC*. Open reading frames (ORFs) are designated 1 through 8, inclusive. ORFs 1 and 3 are overlapping. ORF 1 is a thioesterase (TE); ORF 3 is a methyltransferase. ORF 4 is a hydrolase. ORF 6 is an epoxide hydrolase.

Figure 2 provides a structure of the polyketide tombamycin produced by the TmbA PKS in monomeric and dimeric form. R is a substituted or unsubstituted C₃-C₈ alkyl or cyclic alkyl.

Detailed Description of the Invention

The present invention provides recombinant DNA vectors that comprise all or a portion of any of the genes in the *tmbA* gene cluster. The *tmbA* gene cluster is comprised of PKS genes *tmbA*, *tmbB*, and *tmbC*, and ORFs 1 - 8, inclusive. Each PKS gene in the cluster is composed of one or more PKS modules, each comprising an acyltransferase (AT), ketosynthase (KS), and acyl carrier

protein (ACP) domains and optionally one or more ketoreductase (KR), dehydratase (DH), and enoylreductase (ER) domains as well as linkers that connect one domain to another and one module to another. The boundaries of each of these domains can be identified by sequence comparison with known PKS genes and enzymes. In one important embodiment, the invention provides recombinant DNA vectors that encode all or a portion of one or more of these domains that are useful in the construction of hybrid PKS genes and enzymes.

The sequence of the *tmbA* gene cluster is shown below.

CTCCAGATCGACCTGCATGATCTTGCCGACGAGCTGCAGCAGCTCAGGGTCCTCCTGGAT
CACACGATCGAACGCCCCGTACCTCTGCTGTACACGCCGAGGAACCTCGCTCGCGGGGAG
GTGAGAGATCGGCGGCAGGCGGGCGGGTACGTGAGGGGCTCTGCTCGCGGCGACGACCAT
GCATGTCGGCTCGATCCACGGCGCCTGCGGAGAGAACGGGCCCATTCTGAAGGCCATCAG
CGCTCCCAGGCTGTATCCAAAACACGCGAACGGCAGGTCGAGCATGTCATCCAGCCCCCG
CTCGATGCCGTCGATGAACTCCGACATCGAGCGAGGTTGCTTCTCCTTGGACCGAGCGAA
TCGCCCCGGAGGCTCTATCGGGCAGACGTCGATATGCGCCGGTAGGCTCTTCGCCCAGTC
TCGATATATGGCCCCGCCGGCGCCGGCGTAGGGGAAGCAGAAAAGGCGAAGCTTCGCGTC
CGTCCGCGCGGTCCAGTGCGACAGCCAGGGATTCTTCTCCATGTAGACCTCCGGTGCAAG
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CGCGTCAACCGGCTCCGTGAGAGGGCGATACGACCTCACGGCGAAGGCGGCATCCCGATGC
CGGCGCCACGACCAATGGGGCGCCGATTCTATCCATCCAGCCTCGCACACGATTTTCGATG
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AGACATCGCCATGAACACGCGAAAAATTCGTATCGTACTGTATGCCGCGCTCACGCTGAT
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orf2a bases 791-1144

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orf2b bases 1233-760

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orf 4 bases 3456-2248

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TmbA

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1. 1990-1991		2. 1992-1993		3. 1994-1995		4. 1996-1997		5. 1998-1999		6. 2000-2001		7. 2002-2003		8. 2004-2005		9. 2006-2007		10. 2008-2009		11. 2010-2011		12. 2012-2013		13. 2014-2015		14. 2016-2017		15. 2018-2019		16. 2020-2021		17. 2022-2023		18. 2024-2025		19. 2026-2027		20. 2028-2029		21. 2030-2031		22. 2032-2033		23. 2034-2035		24. 2036-2037		25. 2038-2039		26. 2040-2041		27. 2042-2043		28. 2044-2045		29. 2046-2047		30. 2048-2049		31. 2050-2051		32. 2052-2053		33. 2054-2055		34. 2056-2057		35. 2058-2059		36. 2060-2061		37. 2062-2063		38. 2064-2065		39. 2066-2067		40. 2068-2069		41. 2070-2071		42. 2072-2073		43. 2074-2075		44. 2076-2077		45. 2078-2079		46. 2080-2081		47. 2082-2083		48. 2084-2085		49. 2086-2087		50. 2088-2089		51. 2090-2091		52. 2092-2093		53. 2094-2095		54. 2096-2097		55. 2098-2099		56. 2100-2101		57. 2102-2103		58. 2104-2105		59. 2106-2107		60. 2108-2109		61. 2110-2111		62. 2112-2113		63. 2114-2115		64. 2116-2117		65. 2118-2119		66. 2120-2121		67. 2122-2123		68. 2124-2125		69. 2126-2127		70. 2128-2129		71. 2130-2131		72. 2132-2133		73. 2134-2135		74. 2136-2137		75. 2138-2139		76. 2140-2141		77. 2142-2143		78. 2144-2145		79. 2146-2147		80. 2148-2149		81. 2150-2151		82. 2152-2153		83. 2154-2155		84. 2156-2157		85. 2158-2159		86. 2160-2161		87. 2162-2163		88. 2164-2165		89. 2166-2167		90. 2168-2169		91. 2170-2171		92. 2172-2173		93. 2174-2175		94. 2176-2177		95. 2178-2179		96. 2180-2181		97. 2182-2183		98. 2184-2185		99. 2186-2187		100. 2188-2189		101. 2190-2191		102. 2192-2193		103. 2194-2195		104. 2196-2197		105. 2198-2199		106. 2200-2201		107. 2202-2203		108. 2204-2205		109. 2206-2207		110. 2208-2209		111. 2210-2211		112. 2212-2213		113. 2214-2215		114. 2216-2217		115. 2218-2219		116. 2220-2221		117. 2222-2223		118. 2224-2225		119. 2226-2227		120. 2228-2229		121. 2230-2231		122. 2232-2233		123. 2234-2235		124. 2236-2237		125. 2238-2239		126. 2240-2241		127. 2242-2243		128. 2244-2245		129. 2246-2247		130. 2248-2249		131. 2250-2251		132. 2252-2253		133. 2254-2255		134. 2256-2257		135. 2258-2259		136. 2260-2261		137. 2262-2263		138. 2264-2265		139. 2266-2267		140. 2268-2269		141. 2270-2271		142. 2272-2273		143. 2274-2275		144. 2276-2277		145. 2278-2279		146. 2280-2281		147. 2282-2283		148. 2284-2285		149. 2286-2287		150. 2288-2289		151. 2290-2291		152. 2292-2293		153. 2294-2295		154. 2296-2297		155. 2298-2299		156. 2300-2301		157. 2302-2303		158. 2304-2305		159. 2306-2307		160. 2308-2309		161. 2310-2311		162. 2312-2313		163. 2314-2315		164. 2316-2317		165. 2318-2319		166. 2320-2321		167. 2322-2323		168. 2324-2325		169. 2326-2327		170. 2328-2329		171. 2330-2331		172. 2332-2333		173. 2334-2335		174. 2336-2337		175. 2338-2339		176. 2340-2341		177. 2342-2343		178. 2344-2345		179. 2346-2347		180. 2348-2349		181. 2350-2351		182. 2352-2353		1	
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TmbB

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orf 7

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CGCCAGATCACCGAGATCATGAGGCGCAATTACGAGCAAGCTTACCCCGCGGGCGCCTCG
CCCGCGCTCCGCGGGGTGCACCCGAAGTCGCACGGCTGCGTGAGGGCCCACTTCGTGCTC
GACGAGGGCTTGCCCCGCGAGCTCCGCCATGGCGTCTTCGGGAGCCGCGCGTCTACCCG
GCGTGGGTCCGGTTCTCGTCGACCTCCTCGCGCGTCCAGTCGGACATGAAGCGCGACTCC

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CGCTGCATGGCGATCAAGCTGCTCGGCGTCGAGGGGGAGAAGATCCTCGACGGCGAGAAG
GACGCGACGACGACGAGGATTTCTCTGATGGGGAACACGGACGTGTTCTTCTCGCGGAACATC
GCGGACTACGTCGAGCTCATGTCTGCCATGAGCGCGGGCAAGCCCCCTCTCGTATTTCTGC
TCGCTCCGCCCCGCCCCGCTCCGGCTGCGCGAGCTCATGAACTACCTCTCCGTCTGTGCTC
AAGCCGGTGAAGAACCCCTGCACGCCCCGGTATTTTCAGCCAGACGCCGTTCGGGCTCGGC
GCGCGGGCGATGAAGTTCTGCGTGGTCCCCCGGCCCTGCGGCGCGCCCGGCGTCGGGGTC
GAGCCGGGCGACGACGCGCTCAAGCAGGCGGTGGCGCGGCAGCTCGAGGGGGGAGACTGG
ATGTTCTGACTTCCTCGTGCAGCTCCAGGCCACCCGACGAAGACGCCCATCGAGGATCCG
ACGATTCGCTGGAGCGAGGAACTGTGCGCGTTACCAAGGTCGCGACGATGGTCATCCCG
GCGCAGCGCCTCGACCTCCCGGGACAGGCGGAGTTCGAGGAGAACCTCTCGTTCACGCCC
TGGCACGCCCTGCCGGCGCACCGGCCGCTCGGCGGGCTGAACCGCGCCCCGGCGCGCGGT
TACGAGGCGATATCGAAGCTCCGCCACGAGAGGAACGGGGCTCGCCGCGAGGAGCCGGCG
GCGCCGCGCGCCGGGCGCGCGCGCGCGCCGTCGAGCGCAGGGCCGGCGCGGCTCGGACTC
GGGTGA

MNTTLKLHEEYPPPGEEDSIRQITEIMRRNYEQAYPAGASPALRGVHPKSHGCVRAHFVV
DEGLPRELRHGVFREPRVYPAWVRFSSSTSSRVQSDMKRDSRCMAIKLLGVEGEKILDGEK
DATTDQDFLMGNTDVFFSRNIADYVELMSAMSAGKPLSYFCSLRPPRLRLRELMNYLSVVL
KPVKNPLHARYFSQTPFRLGARAMKFCVVP RPCGAPGVGVEPGDDALKQAVARQLEGGDW
MFDLVQLQAHPTKTPIEDPTIRWSEELSPFTKVATMVI PAQRLDLPQAEFEENLSFTP
WHALPAHRPLGGLNRARRAVYEAISKLRHERNGARREEPAAPRAGRARAPSSAGPARLGL
G

orf 8 - partial sequence

CGGCCGCAATTAACCCTCACTAAAGGGATCATGCTCACTGCGAGCCTGTTCGTGAGCGCG
GTCGTGCAGGTGATCGTCAACGTTCGGACGCTACCGTTCTGCGTCCTCGTCCACATGCTG
CGGTTCGCTCATGCACGGCTTCCGCGCGCTCCACAAGGACCCCGACGTCGTCGATCCGGCG
ACCGGGCGCCCCGGCGCATGGCGCGCTCGCCAGGAACGAAGCCTGTGAAATGCGCTTCGTG
CACGAGGTGCTCTCCGATCCGGTCTGACGCGCGCAGCGCGAAGCTCGCGTACGGCTCG
GACCCCGAGCGGCTCGCGGGCCTCGTCGAGTACATCGACGTCGAGGACCTGATCAGCATC
GCCACCGGCGCCTCGGCGCCGGCCGACGGGGACGGGCGCGAGCTCCTCTTGCCGAGCCGC
TGGGTGAGCTGCGGCCACGGCGGCTCCAGCGCGCCGAAGCGCTACGCGACGCTGGAGCAG
TTCTCCACGTACGTGCGCCGCTGGTTTCCCACCCCTCGAGGGCACGGCGTCGCAGACGTTC
AAGCTGCGCGTCCGGCGGCTGACGCTCCTCGTCTCGATGATGGTGGTCATCCTCTTCAAC
TTCGACGGCTTCCAGGTGCTCGCGCGGCTCCACCAGAGCGGCGCCGCGCGCGCAGGTG
GCCGCCAGGCGGACACGGTGGCGACCACCGCGGCGCGCCTGGGCGAGCTCCCGGCCGGC
GCGCCGGCCGAGCTCCCCGACGCCACGCTCGAGGAGCTCGGGCTCGAGATTCAGAAGACG
GCGACCTCCTCGACGAGGCGAACCTCGGCGTCCGGTGGCAGCAGAGCTGGATCGTCCAG
CGGTTCGCGCGTACCGCCACGATATCCTGGCGCCCCCGCCGACCTCGCTGGAGCTCCTC
CAGGACACGCTGTTCTGGCTGGCCGGGCTCGCCTTCTCGTGGGGTTGCTCTCCCTGGGA
GCGCCCTTCTGGGTACACAGTTTCGCTCGGCTCATCCAGATGCGCAACGAGGTGCAGCAC
CGGAAGCGCCAGGAGAGCGCGTCCGGCGTGAAGGTCGCGAGCACCGCCCTGCCGTTCCTC
GCGCGGAGCGCGCCGTCGCCAAGCCTTCCTGA

RPQLTLTKGIMLTASLFVSAVVQVIVNVGRYRSCVLVHMLRSLMHGFRLHKDPDVVDP
TGRPAHGALARNEACEMRFVHEVLSDPVLHARSAYGSDPERLAGLVEYIDVEDLISI
ATGASAPADGDGREL LPSRWVSCGHGGSSAPKRYATLEQFSTYVRRWFPTLEG TASQTF
KLRVRRLTLLVSMMVVILFNFDGFQVLARLHQSGAARAQVAAQADTVATTAARLGELPAG
APAE LPDATLEELGLEIQKTATLLDEANLGVGWQQSWIVQRF RAYRHDILAPPPTSLELL

QDTLFWLAGLAFSCGLLSLGAPFWVTTFARLIQMRNEVQHRKRQESASGVKVASTALPFP
ARERAVAKPS

The genes of the *tmbA* gene cluster can be isolated from the cosmids of the invention shown in Figure 1 or from *Sorangium cellulosum* genomic DNA.

The gene products of the *tmbA* cluster can be used to synthesize the polyketide tombamycin, the structure of which is shown in Figure 2.

Tombamycin can be dimerized to produce the dimeric form of tombamycin, the structure of which is also shown in Figure 2.

The invention having now been described by way of written description those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.